

Antibiotic Resistance Pattern of Community Acquired Uropathogens at a Tertiary Care Hospital in Jaipur, Rajasthan

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ABSTRACT

Background: Urinary tract infections (UTIs) are amongst the most common infections described in outpatients setting. **Objectives:** A study was conducted to evaluate the uropathogenic bacterial flora and its antimicrobial susceptibility profile among patients presenting to the out-patient clinics of a tertiary care hospital at Jaipur, Rajasthan. **Materials and Methods:** 2012 consecutive urine specimens from symptomatic UTI cases attending to the outpatient clinics were processed in the Microbiology lab. Bacterial isolates obtained were identified using biochemical reactions. Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method. Extended spectrum beta lactamase (ESBL) production was determined by the double disk approximation test and the Clinical and Laboratory Standards Institute (formerly NCCLS) confirmatory method. **Results:** Pathogens were isolated from 346 (17.16%) of the 2012 patients who submitted a urine sample. *Escherichia coli* was the most frequently isolated community acquired uropathogen accounting for 61.84% of the total isolates. ESBL production was observed in 23.83% of *E. coli* strains and 8.69% of *Klebsiella* strains. With the exception of Nitrofurantoin, resistance to agents commonly used as empiric oral treatments for UTI was quite high. **Conclusion:** The study revealed *E. coli* as the predominant bacterial pathogen for the community acquired UTIs in Jaipur, Rajasthan. An increasing trend in the production ESBLs among UTI pathogens in the community was noted. Nitrofurantoin should be used as empirical therapy for primary, uncomplicated UTIs.

Keywords: Urinary tract infection, outpatients, *Escherichia coli*

Introduction

Urinary tract infections (UTIs) are a major public health problem in terms of morbidity and financial cost, and incur the highest total health care cost among urological diseases, exceeding that of chronic renal failure even when renal dialysis and renal transplantation are included.⁽¹⁾ UTI represents one of the most common diseases encountered in medical practice today with an estimated 150 million UTIs per annum worldwide.⁽²⁾

Although UTIs occur in both men and women, clinical studies suggest that the overall prevalence of UTI is higher in women. Uncomplicated UTIs in healthy women have an incidence of 50/1000/year.⁽³⁾ An estimated 50% of women experience at least one episode of UTI at some point in their lifetime and between 20% and 40% of women have recurrent episodes.^(4,5) Approximately 20% of all UTIs occur in men.⁽⁶⁾

Most episodes of UTI are caused by *Escherichia coli* (up to 85%) and *Staphylococcus saprophyticus* (up to 10%), while *Klebsiella pneumoniae* and *Proteus* species account for most of the remaining infections.⁽⁷⁾

The introduction of antimicrobial therapy has contributed significantly to the management of UTIs. However the main problem with current antibiotic therapies is the rapid emergence of antimicrobial resistance in hospitals and the community.⁽⁸⁾ The resistance pattern

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of community acquired uropathogens has not been extensively studied in the Indian subcontinent.⁽⁹⁻¹¹⁾ No data concerning the antimicrobial resistance of bacteria isolated from UTIs from this part of the country (i.e. the state of Rajasthan) has been documented till date. It is important to realize that there may be marked differences between various geographic areas within a vast country like India. Since most UTIs are treated empirically the selection of antimicrobial agent should be determined not only by the most likely pathogen but also by its expected susceptibility pattern. Thus, knowledge of local antimicrobial susceptibility patterns of common uropathogens is essential for prudent empiric therapy of community acquired UTIs.

It was against this backdrop that the current study was undertaken to assess the most frequent pathogens responsible for UTIs in outpatients and their antimicrobial resistance pattern in Jaipur, Rajasthan, through consecutive urine samples collected during two and a half year period from July 2007-December 2009. Additionally, the study also aimed at identifying possible resistance trends.

Materials and Methods

Setting

The present study was conducted at Fortis Escorts Hospital, Jaipur, a 150 bedded tertiary care hospital functional since the mid of the year 2007 and located in the state of Rajasthan. The study was conducted after due approval from the institutional review committee.

Analysis

A retrospective study of all pathogens isolated from urine specimens of patients who attended the out patient departments (OPDs) of our hospital with suspected UTI between July 2007 and December 2009 was conducted. Patients with a history of inpatient admission a week prior to their presentation in our OPDs were excluded from the study to rule out hospital acquired infections. Only one specimen per patient was included. Only patients with significant bacteriuria ($>10^5$ cfu/ml) were included for the microbiological analysis.

Methodology

Freshly voided, midstream urine samples collected in sterile containers from OPD patients were received in the Microbiology lab. The sample was inoculated for semi-quantitative culture on Cystine-Lactose-Electrolyte-Deficient (CLED). CLED media using a calibrated loop. The culture plate was incubated at 37°C for 18-24 hrs under aerobic conditions. Identification of bacterial growth was determined by Gram's staining and standard microbiology techniques.⁽¹²⁾

Antibiotic sensitivity testing

Antibiotic susceptibility was performed by the Kirby-Bauer disc diffusion method on Mueller Hinton agar.⁽¹³⁾ The following antibiotics were tested: Amoxiclav (20/10 mcg), ampicillin (10 mcg), amikacin (30 mcg), trimethoprim/sulphamethoxazole (co-trimoxazole) (25/23.75 mcg), norfloxacin (5 mcg), nalidixic acid (30 mcg), ciprofloxacin (5 mcg), nitrofurantoin (300 mcg), gentamicin (10 mcg), cefuroxime (30 mcg), ceftriaxone (30 mcg), ceftazidime (30 mcg), cephalexin (30 mcg), netilmicin (30 mcg), piperacillin/tazobactam (100/10 mcg), cefoperazone/sulbactam (75/30 mcg), and vancomycin (30 mcg). Dehydrated media and antibiotic discs were procured from Himedia, India. The controls strains used were *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* 25922.

Tests for extended spectrum beta lactamases production

Double disc approximation test

The organism was swabbed on to a Mueller-Hinton agar plate. Antibiotic discs of amoxicillin/clavulanic acid (20/10 mcg) and a cephalosporin disc (cefotaxime (30 mcg), ceftriaxone (30 mcg), and ceftazidime (30 mcg)) were placed at a distance of 15 mm apart and incubated. Organism that showed a clear extension of any of the cephalosporin inhibition zone towards the disc containing clavulanate was considered as ESBL producer.⁽¹⁴⁾

National committee for clinical laboratory standards confirmatory test

While performing antibiotic testing, ceftazidime (30 mcg), and ceftazidime plus clavulanic acid (30/10 mcg) were placed on Mueller-Hinton agar and incubated. Organism was considered as ESBL producer if there was a ≥ 5 mm increase in the zone diameter of ceftazidime/clavulanate disc and that of ceftazidime disc alone. *E. coli* ATCC 25922 and a known in-house ESBL producer were used as negative and positive controls, respectively.⁽¹⁵⁾

Results

During the study period, 2012 consecutive urine samples were processed from OPD patients, 346 (17.19%) of these urine samples yielded significant growth of pathogen. Remaining 1666 samples had either non-significant bacteriuria or had a very low bacterial count or were sterile urine.

Table 1 outlines the demographic profile of patients with community acquired UTIs. The patients were between the ages 0 and 88 years with a mean age of 45.81 years. UTIs were reported in 130 (37.67%) males and 216 (62.42%) females. Females of the reproductive age group (between 21 and 50 years) constituted 41.32%

of the total patients with UTI. However, elderly (61 years or more) males had a higher incidence of UTI (47.28%) compared to the elderly females (21.86%).

Table 2 describes the year wise frequency and distribution of various urinary pathogens isolated from OPD patients. *E. coli* was the predominant uropathogen all through the study period accounting for 61.84% of the total isolates. These were followed by *Enterococcus* species (9.24%) and *Klebsiella* species (6.64%). Gram positive cocci and non-fermenter gram negative bacteria (*Pseudomonas aeruginosa*, *Acinetobacter* spp, etc) accounted for 14.16% and 5.45% of the total isolates, respectively.

Table 3 depicts the frequency of isolation of ESBL producing organisms over the 2½ year period. An increasing trend in the isolation of ESBL producing *E. coli* (from 9.52% to 30.08%) has been noticed over the study period. Extended spectrum beta lactamases production was observed in 23.83% of *E. coli* strains and 8.69% of *Klebsiella* strains.

Table 4 depicts the antibiotic resistance pattern of tested antimicrobials against the encountered uropathogens. A high proportion of strains of *E. coli* were resistance to orally administered drugs such as ampicillin (>80%), amoxiclav (>80%), co-trimoxazole (>67%), nalidixic acid (>95%), norfloxacin (>77%), and ciprofloxacin (>74%). Nitrofurantoin is the drug with least resistance (>5-6%) to *E. coli* throughout the 2½ years study period. However, this drug demonstrated a very high resistance to non-fermenters and gram negative enteric bacteria other than *E. coli* and a low resistance (>5%) to gram positive cocci. Aminoglycosides (amikacin, gentamicin, and netilmicin) have also shown a decreasing resistance trend against *E. coli* from the year 2007 to 2009 with amikacin being the least resistant aminoglycoside. Cefoperazone/sulbactam and piperacillin/tazobactam have emerged as least resistant drugs to *E. coli* in the year 2009. The overall resistance rates of 6.19% and 9.09% to piperacillin/tazobactam and cefoperazone/sulbactam respectively were observed for *E. coli* strains. Vancomycin remains the drug with least resistance for gram positive cocci. However, vancomycin resistance was noted in 5% of gram positive cocci (which included the *Enterococcus* species) in the year 2009. Non-fermenter gram negative bacteria have shown a high degree of resistance to all the classes of drugs tested with least resistance to piperacillin/tazobactam (46.67%).

Discussion

This is the first study to evaluate the susceptibility patterns of bacterial strains isolated from community acquired UTIs in Jaipur, Rajasthan. This study provides valuable laboratory data and allows comparison of the situation in Rajasthan with other parts of the country.

Table 1: Age and sex distribution of patients presenting to the OPDs with urinary tract infections

Age group (in years)	Females No. (%)	Males No. (%)
0-10	17 (7.87)	19 (14.6)
11-20	9 (4.16)	7 (5.38)
21-30	53 (24.53)	3 (2.30)
31-40	30 (13.88)	5 (3.84)
41-50	25 (11.57)	17 (13.07)
51-60	35 (16.20)	18 (13.84)
61-70	31 (14.35)	23 (17.69)
71-80	13 (6.01)	24 (18.46)
81-90	3 (1.38)	14 (10.76)
Total	216 (62.42)	130 (37.67)

OPD: Outpatient Department

Table 2: Year wise distribution of microbiological flora causing urinary tract infections in OPD patients

Microorganism	2007 isolates No. (%)	2008 isolates No. (%)	2009 isolates No. (%)	Total isolates No. (%)
<i>Escherichia coli</i>	21 (58.33)	80 (57.97)	113 (65.69)	214 (61.84)
<i>Klebsiella</i> spp.	01 (2.77)	05 (3.62)	17 (9.88)	23 (6.64)
<i>Proteus</i> spp.	01 (2.77)	01 (0.72)	03 (1.74)	05 (1.44)
<i>Enterobacter</i> spp.	-	07 (5.04)	06 (3.48)	13 (3.75)
<i>Citrobacter</i> spp.	-	04 (2.89)	04 (2.32)	08 (2.31)
<i>Morganella morganii</i>	-	02 (1.44)	01 (0.58)	03 (0.86)
<i>Pseudomonas</i> spp.	-	09 (6.52)	07 (4.06)	16 (4.62)
<i>Acinetobacter</i> spp.	-	01 (0.72)	-	01 (0.28)
<i>Burkholderia cepacia</i>	-	01 (0.72)	-	01 (0.28)
<i>Stenotrophomonas maltophilia</i>	-	01 (0.72)	-	01 (0.28)
<i>Enterococcus</i> spp.	-	16 (11.59)	16 (9.30)	32 (9.24)
<i>Coagulase negative Staphylococci</i>	-	-	03 (1.74)	03 (0.86)
<i>Staphylococcus aureus</i>	12 (33.33)	06 (4.34)	01 (0.58)	19 (5.49)
<i>Streptococcus</i> species	-	05 (3.62)	01 (0.58)	06 (1.73)
<i>Candida</i> species	01 (2.77)	-	-	01 (0.28)
Total	36	138	172	346

OPD: Outpatient Department

Table 3: Percentage distribution of extended spectrum beta-lactamase producing uropathogens isolated over the study period

Year	Total no. of <i>E. coli</i> isolated	ESBL producing <i>E. coli</i> No. (%)	Total no. of <i>Klebsiella</i> spp. isolated	ESBL producing <i>Klebsiella</i> species No. (%)
2007	21	2 (9.52)	1	0
2008	80	15 (18.75)	5	2 (40)
2009	113	34 (30.08)	17	0
Total	214	51 (23.83)	23	2 (8.69)

OPD: Outpatient Department

The results show that 17.19% of urine samples from patients attending the outpatient clinics at our hospital yielded significant pathogens. The culture positive rate for community acquired uropathogens was higher in our study than that reported at Aligarh, India (10.86%)⁽⁹⁾ and at Tehran, Iran (6.3%).⁽¹⁶⁾

Table 4: Year wise representation of the antibiotic resistance profile of various uropathogens to antimicrobials tested

Organism	Year	No. of isolates	Amp	Amc	Amk	Net	Gen	Cxm	Cft	Ctr	Caz	Nor	Cip	Ntf	Na	Sxt	Pip/taz	Cfz/Sul	Van
			R%	R%	R%	R%	R%	R%	R%	R%	R%	R%	R%	R%	R%	R%	R%	R%	R%
<i>E. coli</i>	2007	21	83.33	76.47	10.53	15.79	31.58	76.47	68.75	66.67	66.67	94.44	82.35	5.56	100	72.22	26.67	14.29	-
<i>E. coli</i>	2008	80	80.33	81.08	11.84	12.86	30.67	65.57	68	65.33	64.94	76.62	74.32	5.19	96	64.47	8.82	8.11	-
<i>E. coli</i>	2009	113	83.33	81.08	5.36	0	25.66	50	71.56	72.07	72.32	76.11	73.87	6.19	92.92	69.37	1.8	0	-
Total		214	81.18	80.69	8.21	13.48	28.02	66.67	70	69.15	69.08	77.88	74.75	5.77	94.63	67.8	6.19	9.09	-
GNEB	2007	2	100	100	100	50	100	100	100	100	100	50	50	50	50	100	50	100	-
GNEB	2008	19	84.62	50	31.25	35.71	53.33	76.92	75	81.25	75	62.5	58.82	50	75	75	20	11.76	-
GNEB	2009	31	100	85.71	33.33	45.16	40	100	61.29	58.62	60	51.61	51.61	67.74	54.84	54.84	31.03	26.67	-
Total		52	88.89	75.47	35.42	42.55	46.81	84.21	67.35	68.09	66.67	55.1	54	61.22	61.22	63.27	28.26	22.92	-
GPC	2007	12	27.27	27.27	60	40	63.64	80	30	22.22	100	70	77.78	0	100	80	33.33	28.57	0
GPC	2008	27	7.69	0	14.81	0	18.52	19.05	36	29.63	56.52	44.44	52	7.41	92.59	25.93	4.55	4	0
GPC	2009	21	50	30	68.75	47.62	45	100	80.95	81.25	80	66.67	70	4.76	100	85.71	15	20	5
Total		60	26.32	16.07	39.62	24.56	36.21	40.63	51.79	44.23	69.44	56.9	62.96	5.17	96.36	56.9	13.73	13.46	1.82
NFGNB	2007	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
NFGNB	2008	12	88.89	88.89	63.64	50	66.67	80	81.82	77.78	72.73	63.64	60	90.91	81.82	80	50	44.44	-
NFGNB	2009	7	0	100	57.14	33.33	85.71	100	100	100	100	85.71	42.86	100	100	100	42.86	71.43	-
Total		19	88.89	90	61.11	42.86	75	81.82	88.89	86.67	83.33	72.22	52.94	94.44	88.89	83.33	46.67	56.25	-

GNEB: Gram negative enteric bacteria other than *E. coli*, GPC: Gram positive cocci, NFGNB: Non-fermenter gram negative bacteria, Amp: Ampicillin, Amc: Amoxycyclav, Amk: Amikacin, netilmycin, Gen: Gentamicin, Cxm: Cefuroxime, Cft: Cephalexin, Ctr: Ceftriaxone, Caz: ceftazidime, Nor: -norfloxacin, Cip: ciprofloxacin, Ntf: Nitrofurantoin, Na: Nalidixic acid, Sxtulphamethoxazole/trimethoprim (co-trimoxazole), Pip/taz: Piperacillin/tazobactam, Cfz/sul: Cefoperazone/sulbactam, Van: Vancomycin, R%- resistance percentage

The demographic data indicates that women of the reproductive age group formed the main group of adult patients with UTI presenting to the outpatient clinics (42.34% of all UTI detected in women of age 21-50 years.) UTIs were reported in 62.42% of females and in 37.67% of males. It has been extensively reported that adult women have a higher prevalence of UTI than men, principally owing to anatomic and physical factors.⁽⁹⁾ Elderly (61 years or more) males had a higher incidence of UTI (49.23%) compared to the elderly females (21.75%). This is probably because with the advancing age, the incidence of UTI increases in men due to prostate enlargement and neurogenic bladder.⁽¹⁷⁾

The study demonstrates that *E. coli* remain the leading uropathogen being responsible for 61.84% of community acquired UTIs in our area, and no change in its prevalence among all community-acquired uropathogens was observed over the 2½ year study period. This is in consistence with findings of other studies around the globe in which *E. coli* was the most frequently reported isolate from patients with community acquired UTIs.^(7,18-21) Following *E. coli*, our study shows *Enterococcus* species (9.24%) and *Klebsiella* species (6.64%) as the other common uropathogens in the community setting. Our findings are in accordance with a study by Dias Neto *et al.*⁽²²⁾ Enterobacteriaceae have several factors responsible for their attachment to the uroepithelium. These gram negative aerobic bacteria colonize the urogenital mucosa with adhesion, pili, fimbriae, and P1-blood group phenotype receptor.⁽¹⁷⁾ In our study, Enterobacteriaceae bacteria accounted for 76.87% of all the isolates, followed by gram positive cocci (14.16%) and non-fermenter gram negative bacteria (5.49%).

Our study reveals 23.83% of the *E. coli* isolates and 8.69% of *Klebsiella* species to be ESBL producers. Pitout *et al.* have also highlighted the emergence of Enterobacteriaceae producing ESBLs in the community particularly from UTIs.⁽²³⁾ Aggarwal *et al.* reported 40% of *E. coli* and 54.54% of *Klebsiella* species to be ESBL producers from Rohtak, Haryana.⁽²⁴⁾ In another study from Nagpur, 18.5% of *E. coli* isolates and 25.6% of *Klebsiella* isolates were found to be ESBL producers.⁽²⁵⁾ This geographical difference may be due to different patterns of antibiotic usage. Our study confirms the global trend towards increased resistance to β -lactam antibiotics. ESBL producing bacteria may not be detectable by routine disk diffusion susceptibility test, leading to inappropriate use of antibiotics and treatment failure. It is emphasized that institutions should employ appropriate tests for their detection and avoid indiscriminate use of third-generation cephalosporins.

Generally, uncomplicated UTIs are treated in the community with short courses of empirical antibiotics. In many cases, urine samples are only sent for microbiological evaluation following treatment failure, recurrent or relapsing infection. Although the levels of resistance we observed amongst community isolates may therefore overestimate the true rate of resistance in the community, the high levels of resistance of gram negative uropathogens to ampicillin, amoxycyclav, and co-trimoxazole raise concerns over the use of these agents. Our findings are in consistence with the recent data reported from other developing countries.^(26,27) Our findings thus suggest that empirical treatment with these drugs should no longer be appropriate.

The high antibiotic resistance against ampicillin and co-trimoxazole could be attributed to their wide usage for a variety of other indications.

Aminoglycosides (amikacin, gentamicin, and netilmicin) have also shown a decreasing resistance trend against *E. coli* from the year 2007 to 2009. Aminoglycosides being injectables are used restrictively in the community-care setting and hence have shown better sensitivity rates.

The overall resistance of *E. coli* to nalidixic acid, norfloxacin, and ciprofloxacin was 94.63%, 77.88%, and 74.75%, respectively. Fluoroquinolones have a wide variety of indications, permeate most body compartments, and are ubiquitously prescribed, accounting for the emergence of their resistance. Our findings indicate that urgent strategies to counteract increased resistance to these drugs must be developed or their use in uncomplicated infections should be strictly curtailed.

Nitrofurantoin has shown the least resistance for *E. coli*. Our findings are similar to other Indian studies which have also demonstrated nitrofurantoin as an appropriate agent for first-line treatment of community acquired UTIs.^(10,11) Given the fact that Nitrofurantoin has no role in the treatment of other infections, it can be administered orally and is highly concentrated in urine; it may therefore be the most appropriate agent for empirical use in uncomplicated UTI. Fosfomycin is another oral antibiotic which is commonly used for treatment of CA-UTI in Europe with low resistance rates; however, it is not marketed in India.⁽¹¹⁾

Resistance to cefoperazone/sulbactam and piperacillin/tazobactam for Enterobacteriaceae was low probably reflecting their lower usage for treatment of community acquired infections. Vancomycin is the drug with least resistance to gram positive cocci. An overall 1.82% resistance for vancomycin was observed in gram positive cocci belonging to the *Enterococcus* species. Although, the frequency of isolation of vancomycin-resistant Enterococci is not very high in our setting as compared to West, this may just be the beginning of the problem.

Conclusion

In our study, the culture positive rate for community-acquired uropathogens was high, with the majority coming from adult female patients. As expected, *E. coli* was the most common etiological agent identified and remains susceptible to nitrofurantoin. This drug should therefore be the ideal antibiotic to use for uncomplicated UTI.

Our findings suggest the presence of ESBL-producing strains in the community; therefore, monitoring of

antibiotic susceptibility of bacterial isolates in the community should be mandatory. International guidelines are no longer applicable for treating community acquired UTIs in India and development of specific guidelines based on local susceptibility patterns are necessary.

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